(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) Publication number : 0 382 381 B1

(12) EUROPEAN PATENT SPECIFICATION

(45) Date of publication of patent specification : 26.05.93 Bulletin 93/21

(51) Int. Cl.[5] : A61K 37/02, A61K 37/36

(21) Application number : 90300840.7

(22) Date of filing : 26.01.90

(54) Treatment of leukocyte dysfunction with GM-CSF.

Consolidated with 90903118.9/0455726 (European application No./publication No.) by decision dated 14.01.92.

(30) Priority : 30.01.89 US 304391

(43) Date of publication of application : 16.08.90 Bulletin 90/33

(45) Publication of the grant of the patent : 26.05.93 Bulletin 93/21

(84) Designated Contracting States : AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(56) References cited :
EP-A- 0 211 684
EP-A- 0 276 846
EP-A- 0 318 184

(56) References cited :
WO-A-87/02060
MEDLINE, abstract no. 90008993; D.F.GRUBER et al.: "Bone marrow myeopoiesis in rats after 10%, 20% or 30% thermal injury"
BIOLOGICAL, abstract no. 89116980; R. NETA et al.: "Cytokines in therapy of radiation injury"

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Description

This invention relates to the treatment of leukocyte dysfunction associated with physical trauma, particularly thermal injury, by administering effective doses of GM-CSF.

GM-CSF is a lymphokine (stimulator of the immune system) that exhibits a broad spectrum of immune cell stimulation as described in Burgess and Metcalf, *Blood*, 56: 947 (1980) and Metcalf, *Blood*, 67: 257 (1986). GM-CSF has been shown to increase the leukocyte count in patients with acquired immunodeficiency syndrome [Brandt et al., *N. Engl. J. Med.*, 318: 869 (1988)] and people suffering from chemotherapy-induced myelosuppression [Antman et al., *New Engl. J. Med.*, 319: 593 (1988), and it has been suggested that various colony stimulating factors alone or in combination with erythropoietin and/or an antiviral agent and/or interleukin-2 (IL-2) may be useful for the treatment of patients suffering from AIDS-type disease (PCT Application No. 87/03204).

Although GM-CSF was identified because of its ability to stimulate proliferation of hematopoietic precursor cells, it is also able to stimulate a number of functional aspects of mature granulocytes and macrophages. These effects include synthesis of biologically active molecules such as prostaglandin E [Hancock et al., *J. Immunol.*, 140: 3021 (1988) and Kurland et al., *Proc. Natl. Acad. Sci. USA*, 76: 2326 (1979)]; increased phagocytic activity [Weisbart et at., *Nature*, 332: 647 (1988)]; expression and/or affinity of various membrane markers such as the IL-2 receptor [Hancock et al., *J. Immunol.*, 140: 3021 (1988)] and the bacterial product formylmethionylleucylphenylalanine receptor on neutrophils, which receptors elicit the production of superoxide anions [Atkinson et al., *Immunology*, 64: 519(1988)]; and the regulation of enzyme activity such as the stimulation of guanylate cyclase and the inhibition of adenylate cyclase [Coffey et al., *J. Immunol.*, 140: 2695 (1988)].

In cases of physical trauma, such as thermal injury, there is an associated dysfunction of the white blood cells (WBC), particularly monocytes and leukocytes. Thus, although there may be sufficient numbers of WBC to function -- i.e., engage in phagocytosis and superoxide generation--if operating normally, because of the leukocyte dysfunction there is a malfunction or suppression of the immune system. The immune system malfunction is attributable to the leukocyte dysfunction rather than to there being an insufficient number of leukocytes.

Those skilled in the art will appreciate that such leukocyte dysfunction jeopordizes the recovery of the physical trauma patient. For example, in the thermal burn patient such dysfunction can result in a greater risk of infection. To date no significant impact has been made in treating leukocyte dysfunction in vivo in thermal injury (burn) patients and the associated clinical consequences such as infection.

A welcome contribution to the art would be a method of treating leukocyte dysfunction associated with physical trauma, particularly thermal injury. Such a contribution is provided by this invention.

The invention may be summarized in the following manner. It has surprisingly and unexpectedly been discovered that leukocyte dysfunction associated with physical trauma, particularly thermal injury, can effectively be treated by the administration of GM-CSF. It has been discovered that GM-CSF administered to thermal injury patients results in increased leukocyte function--i.e., there is a potentiation of leukocyte function. Such treatment therefore results in a significantly higher response to infection in thermal injury patients. The potentiation in vivo of leukocyte function with GM-CSF is to be distinguished from merely increasing the number of dysfunctioning leukocytes. Since leukocyte dysfunction is also present in other types of physical trauma besides thermal injury, it is contemplated that leukocyte dysfunction associated with these other types of physical trauma are likewise treatable with GM-CSF.

This invention also provides the use of GM-CSF for the manufacture of a medicament for use in a method of treating a mammal having leukocyte dysfunction that is associated with physical trauma such as thermal injury by administering to said mammal an effective amount of GM-CSF to potentiate the function of said leukocytes.

Figure 1 is a graphical presentation of enhanced proliferation of monocytes in patients with thermal injury who were treated with GM-CSF.

Figures 2 and 3 are graphical presentations of enhanced oxidative bursts of monocytes in patients with thermal injury who were treated with GM-CSF.

Figures 4 and 5 are graphical presentations showing no significant stimulation of oxidative bursts in monocytes over a period of time from FMLP(formylmethionylleucylphenylalanine) alone.in patients with thermal injury who were treated with GM-CSF.

Preferably the mammals treated will be humans and the GM-CSF utilized will be one of the human allotypes.

In a particularly preferred embodiment an effective amount of the GM-CSF is administered intravenously, such as by injection or infusion, over a time sufficient to allow the GM-CSF to potentiate leukocyte function without significant loss in GM-CSF activity (such as by metabolism of the GM-CSF). In general the effective amount is 3 to 30 micrograms of GM-CSF per kilogram of body weight per day which is administered by intravenous infusion over a time period of 30 minutes to 24 hours. Preferably the effective amount is 3 to 15 micrograms per kilogram of body weight which is administered by intravenous infusion over a time period of 2 to 6 hours with 2 to 4

hours being more preferable and 4 hours being most preferable. Most preferably, the dosages utilized are 3, 10, or 15 micrograms per kilogram of body weight per day. The actual dosage may be varied depending on the patient's weight and tolerance to the GM-CSF.

\Unless stated otherwise, the term "physical trauma" as used herein refers to trauma to the various tissues and organs of the body including organ systems, musculature, the skeletal system, the vascular system, and the like. The trauma may result from any mechanism or mode of action sufficient to cause injury, such as for example, thermal injury (burn), electrical burn, chemical burn, blunt trauma such as that resulting from accident or assault, traumatic amputation, and the like.

Unless stated otherwise, the term "thermal injury" as used herein means the physiological insult to an individual caused by excessive heat, as distinguished from electric and chemical burns.

Unless stated otherwise, the term "leukocyte dysfunction" as used herein means that the leukocytes, e.g., monocytes, have a significantly reduced functional capability or complete failure of their ability to protect the human from overwhelming infection. Some of the functions of monocytes and granulocytes include in vivo or in vivo demonstrations of phagocytosis and/or superoxide generation.

Unless stated otherwise, the term "leukocyte function" as used herein refers to the normal functioning of the leukocytes, e.g., monocytes, in their engagement in phagocytosis and/or superoxide generation.

Unless stated otherwise the term "leukocyte" as used herein has its generally art recognized meaning and therefore includes the different cellular types that are classified as being white blood cells including, for example, cells of the myeloid, lymphoid, and monocytic series.

This invention provides a method for potentiating leukocyte function in dysfunctional leukocytes in mammals, wherein such dysfunction is associated with thermal injury or other forms of physical trauma. In this method an effective amount of GM-CSF is administered over a time period sufficient to effect increased leukocyte.function. In effect, the method of this invention significantly reduces or reverses the dysfunction of the leukocytes.

Any suitable GM-CSF may be employed in the present invention. Complementary DNAs (cDNAS) for GM-CSF have recently been cloned and sequenced by a number of laboratories, e.g. Gough et al., *Nature*, 309: 763 (1984) (mouse); Lee et al., *Proc. Natl. Acad. Sci. USA*, 82: 4360 (1985) (human); Wong et al., *Science*, 228: 810 (1985) (human and gibbon); Cantrell et al., *Proc. Natl. Acad. Sci. USA*, 82: 6250 (1985) (human). Moreover, non-recombinant GM-CSF has been purified from various culture supernatants, e.g. Burgers et al., *Exp. Hematol.*, 9: 893 (1981) (mouse); Sparrow et al., *Exp. Hematol.*, 12: 267 (1984) (rat); Gasson et al., *Science*, 230: 1171 (1985) (human); Burgess et al., *Blood*, 69: 43 (1987) (human). Among the human GM-CSFs, nucleotide sequence and amino acid sequence heterogeneity have been observed. For example, both threonine and isoleucine have been observed at position 100 of human GM-CSF with respect to the N-terminal alanine, suggesting that allelic forms, or polymorphs, of GM-CSF may exist within human populations. Also, various leader sequences may occur at the N-terminal position of the amino acid sequence. These leader sequences may be of various lengths and amino acid composition, which may or may not affect biological activity. Preferably, the GM-CSF used in the present invention for treating humans will be a human GM-CSF (hGM-CSF), most preferably the recombinant human GM-CSF (rhGM-CSF) described in Lee et al., *Proc. Natl. Acad. Sci. USA*, 82: 4360 (1985), as purified in U.S. Patent Application No. 111,886, filed October 23,1987.

According to this invention, mammals are administered an effective amount of GM-CSF. An effective amount is that amount required to potentiate the function of the dysfunctional leukocytes. Preferably, as stated previously, the mammal is a human and the preferred GM-CSF is recombinant human GM-CSF (rhGM-CSF). Generally, an amount of GM-CSF of 3 to 30 micrograms per kilogram of body weight per day is sufficient in most patients to produce the desired potentiation of function in the dysfunctional leukocytes. Preferably 3 to 25 micrograms per kilogram of body weight is administered per day with 3 to 15 micrograms per kilogram being more preferable, and dosages of 3, 10, or 15 micrograms per kilogram being most preferable. An even more preferable dose is micrograms per kilogram.

The GM-CSF is most effective when administered so that there is an effective level of GM-CSF in the blood maintained over a period of time as opposed to rapid administration which results in a sudden increase in GM-CSF blood levels followed by a rapid decrease in GM-CSF blood levels due to metabolism of the GM-CSF. Generally, administration by intravenous bolus and/or infusion over a time period of from 30 minutes to 24 hours is sufficient. Preferably such administration is done over a time period of 2 to 6 hours, more preferably 2 to 4 hours and most preferably 4 hours. The GM-CSF may also be administered intramuscularly, subcutaneously, topically by direct application to an open injury site, transdermally, nasally (nasal spray), orally(oral spray), by insufflation and the like. Thus, any method of administering an effective dose to provide effective blood levels over a period of time is contemplated.

The GM-CSF, preferably rhGM-CSF, may be prepared in any number of conventional dosage forms such as for example, parenteral, including sterile sol-

utions or suspensions; topical dosage forms such as creams, ointments, lotions and transdermal devices (e.g., of the conventional reservoir or matrix patch type).

The formulations and pharmaceutical compositions contemplated by the above dosage forms can be prepared with conventional pharmaceutically acceptable excipients and additives, using conventional techniques.

Presently, the GM-CSF, preferably recombinant human rhGM-CSF, is administered via the intravenous route. The solutions to be administered may be reconstituted from lyophilized powders and they may additionally contain preservatives, buffers, dispersants, etc. Preferably, rhGM-CSF is reconstituted with any isotonic meduim normally utilized for intravenous injection, e.g., preservative-free sterile water. The maximum concentration of rhGM-CSF preferably should not exceed 1500 micrograms per milliliter. Administration may be accomplished by continuous intravenous infusion or by intravenous injection. For continuous infusion, the daily dose can be added to normal saline and the solution infused by mechanical pump or by gravity.

The following examples are illustrative only and should not be construed as limiting the invention in any way. Those skilled in the art will appreciate that variations are possible which are within the spirit and scope of the appended claims.

The effect of GM-CSF on potentiating the function of dysfunctional leukocytes in patients suffering from thermal injury over 20% to 70% of their body surface area can be determined by the following test protocol: Initially patients age 18 or older with thermal injury over 20 to 40% of their body surface area, in whom cardiovascular stabilization has taken place or was ongoing, and without inhalation injury to the lungs, were treated with recombinant human rhGM-CSF within 48 hours of injury. Thereafter patients age 18 or older with thermal injury over 40 to 70% of their body surface area, in whom cardiovascular stabilization has taken place or was ongoing and without inhalation injury, would be treated with rhGM-CSF within 48 hours of injury. Thereafter, patients age 18 or older with thermal injury over 40 to 70% of their body surface area, in whom cardiovascular stabilization has taken place or was ongoing and with mild to moderate inhalation injury (as diagnosed by physical exam on xenon scan) but without bronchoscopic evidence of inhalation injury will be treated with rhGM-CSF within 48 hours of injury. The rhGM-CSF was obtained as described in Lee et al., *Proc. Natl. Acad. Sci. USA*, 82: 4360 (1985) and U.S. Patent Application No. 111,886, filed October 23,1987. The rhGM-CSF was in the form of a lyophilized powder and was prepared for intravenous administration by the attending physician or pharmacist by diluting to 1 ml with sterile water then to this resulting solution there was added 50 ml of normal saline. The patients were more preferably administered rhGM-CSF in doses of 3, 10, or 15 micrograms per kilogram of body weight intravenously (bolus or infusion) over a 2 hr to 4 hour time period, most preferably 4 hours, once a day. Each dose level of GM-CSF was administered to groups of 3 to 5 patients.

Blood samples were taken for in vitro analysis to determine leukocyte function. The blood samples were analyzed by methods known in the art. An increase in white blood cell counts of 50% or more above baseline is indicative of therapeutic efficacy and clinically meaningful results.

The combined results of all the dosage levels are given in Figures 1 to 5. In Figures 1 to 5 "Normal" or "Control" refers to the results with a normal population of patients--i.e., no thermal injury--, "Burn Control" refers to a population of patients with thermal injury and no GM-CSF treatment. In Figures 2 to 5 "Pre" refers to pretreatment--i.e., no administration of GM-CSF--and the numbers "1", "8", and "15" on the x-axis refers to the number of days of treatment with rhGM-CSF.

Figure 1 represents a comparison of tritiated thymidine incorporation in a group of patients in comparison to a historical control group. As noted in Figure 1, in the four patient study(N=4) at greater than ten-day post burn, there was a significant enhancement of proliferation of monocytes as manifested by tritiated thymidine incorporation. There are two controls noted in Figure 1, the far left bar(N=5) indicates normal control with no intracedent illness such as thermal injury and the middle bar(N=7, greater than 15 days post burn) represents a cohort of patients who actually did have thermal injury.

Figures 2 and 3 represents the human oxidate of bursts of PMA stimulation of monocytes. These figures attempt to demonstrate that with continued rhGM-CSF administration over a period of 15 days there is an enhancement of oxidative bursts of monocytes.

Figures 4 and 5 demonstrate FMLP stimulation of human oxidative respiration. These figures demonstrate that over a period of time there is no significant stimulation of oxidative bursts in monocytes from FMLP alone.

Claims

1. The use of GM-CSF for the manufacture of a medicament for use in a method of treating a mammal having leukocyte dysfunction that is associated with physical trauma by administering to said mammal an effective amount of GM-CSF to potentiate the function of said leukocytes.

2. The use claimed in Claim 1 wherein said GM-CSF

is recombinant human GM-CSF.

3. The use claimed in Claim 1 or Claim 2 wherein the medicament is in a form suitable for administration by intravenous infusion or injection.

4. The use claimed in any preceding claim wherein the physical trauma is thermal injury.

Patentansprüche

1. Verwendung von GM-CSF für die Herstellung eines Medikaments zur Verwendung in einer Behandlungsmethode bei Säugern, die eine Leukocyten-Dysfunktion haben, die mit einem körperlichen Trauma verbunden ist, durch das Verabreichen einer wirksamen Menge von GM-CSF an den Säuger, um die Funktion der Leukocyten zu potenzieren.

2. Verwendung nach Anspruch 1, worin das GM-SCF rekombinantes humanes GM-CSF ist.

3. Verwendung nach Anspruch 1 oder 2, worin das Medikament in einer für die Verabreichung durch intravenöse Infusion oder Injektion geeigneten Form vorliegt.

4. Verwendung nach irgendeinem der vorangehenden Ansprüche, worin das körperliche Trauma eine thermische Schädigung ist.

Revendications

1. Utilisation de GM-CSF pour la fabrication d'un médicament à utiliser dans une méthode de traitement d'un mammifère présentant un disfonctionnement leucocytaire qui est associé à un traumatisme physique par administration, audit mammifère, d'une quantité efficace de GM-CSF pour potientaliser la fonction desdits leucocytes.

2. Utilisation selon la revendication 1, où GM-CSF est GM-CSF humain recombinant.

3. Utilisation selon la revendication 1 ou la revendication 2, où le médicament est sous une forme appropriée pour une administration par infusion ou injection intraveineuse.

4. Utilisation selon toute revendication précédente, où le traumatisme physique est une blessure thermique.

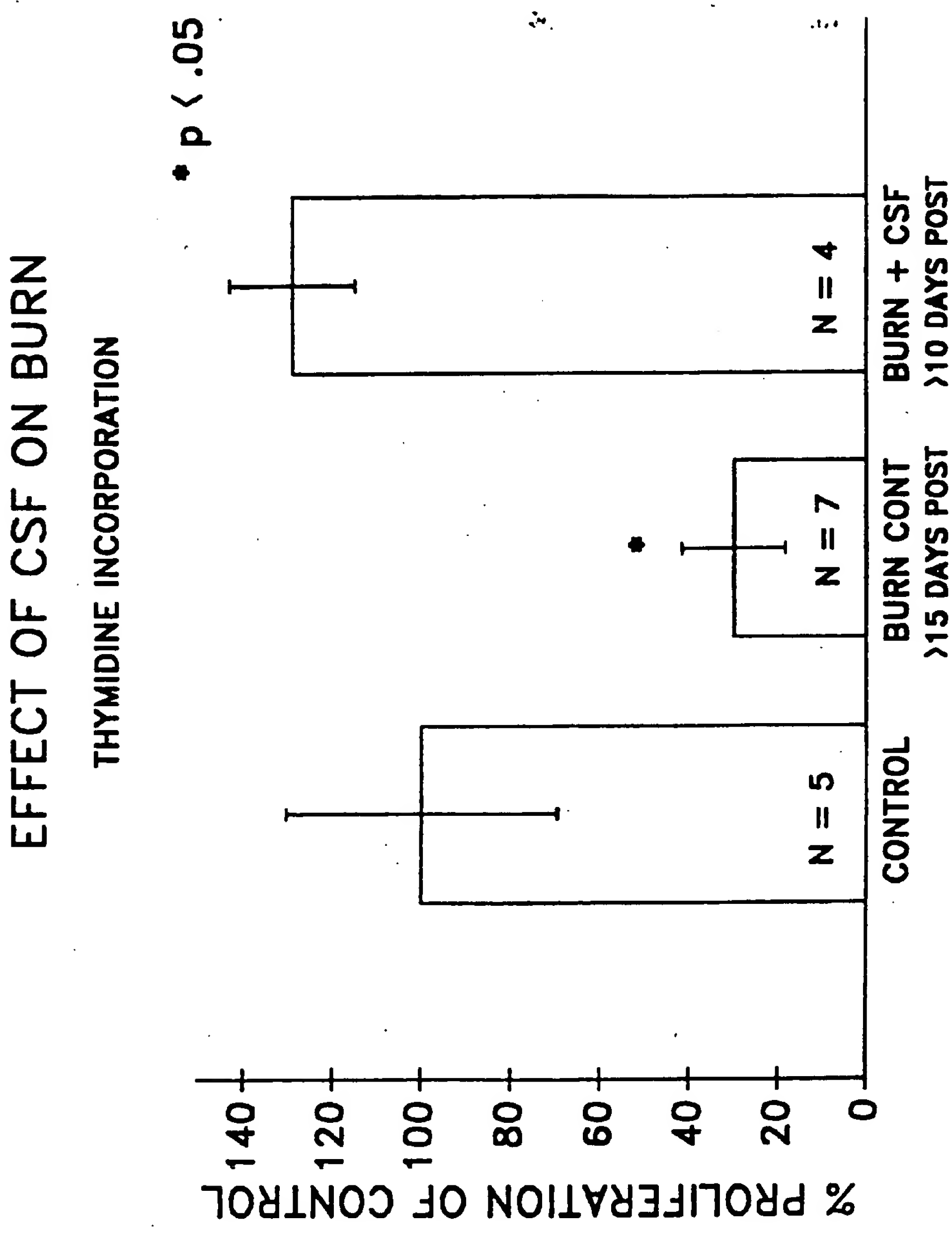
EFFECT OF CSF ON BURN
THYMIDINE INCORPORATION
* p < .05
% PROLIFERATION OF CONTROL
140
120
100
80
60
40
20
0
N = 5
N = 7
N = 4
*
CONTROL
BURN CONT
>15 DAYS POST
BURN + CSF
>10 DAYS POST

Figure 1

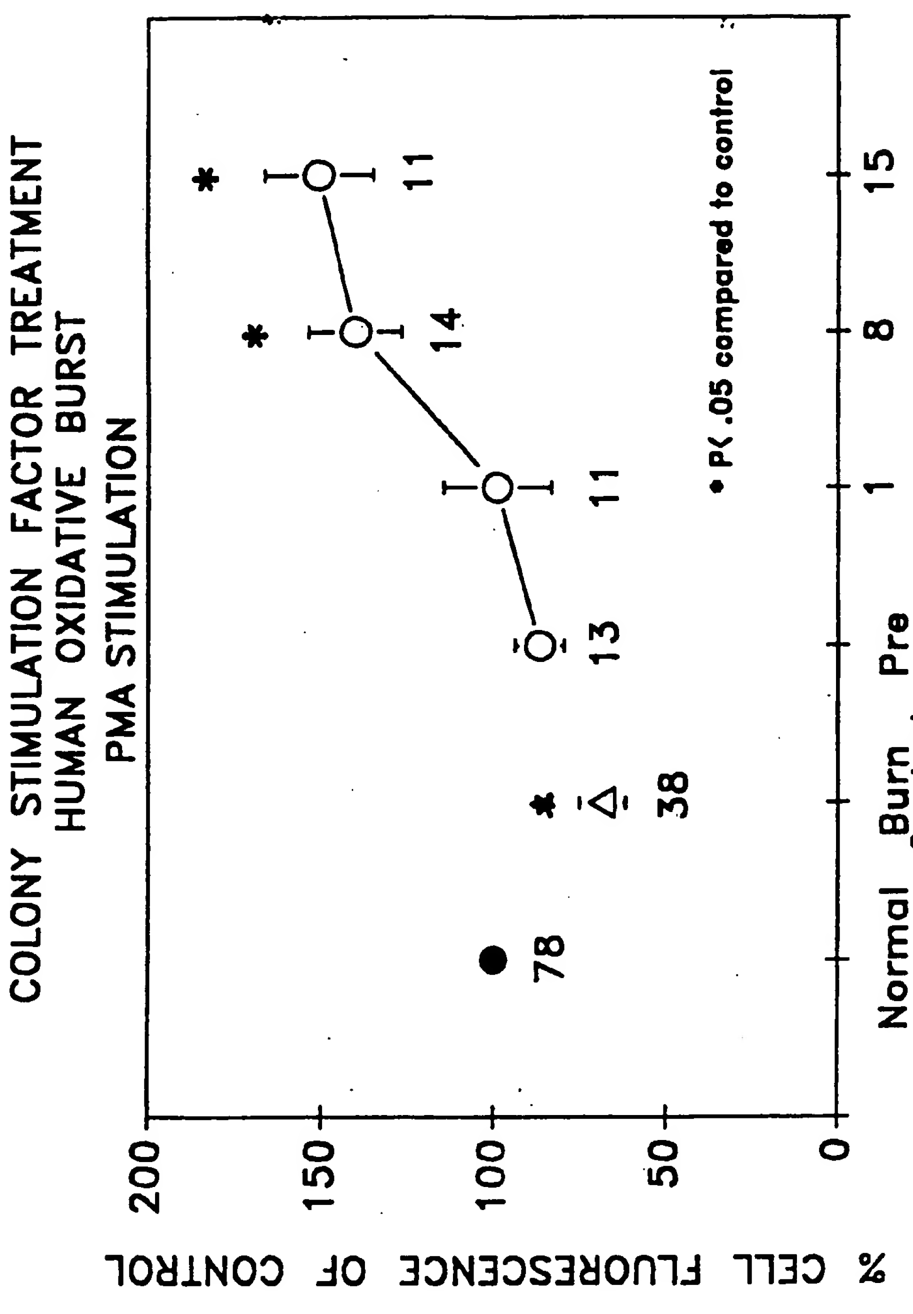
COLONY STIMULATION FACTOR TREATMENT
HUMAN OXIDATIVE BURST
PMA STIMULATION
% CELL FLUORESCENCE OF CONTROL
200
150
100
50
0
Normal
Burn Control
Pre
1
8
15
78
38
13
11
14
11
* P< .05 compared to control

Figure 2

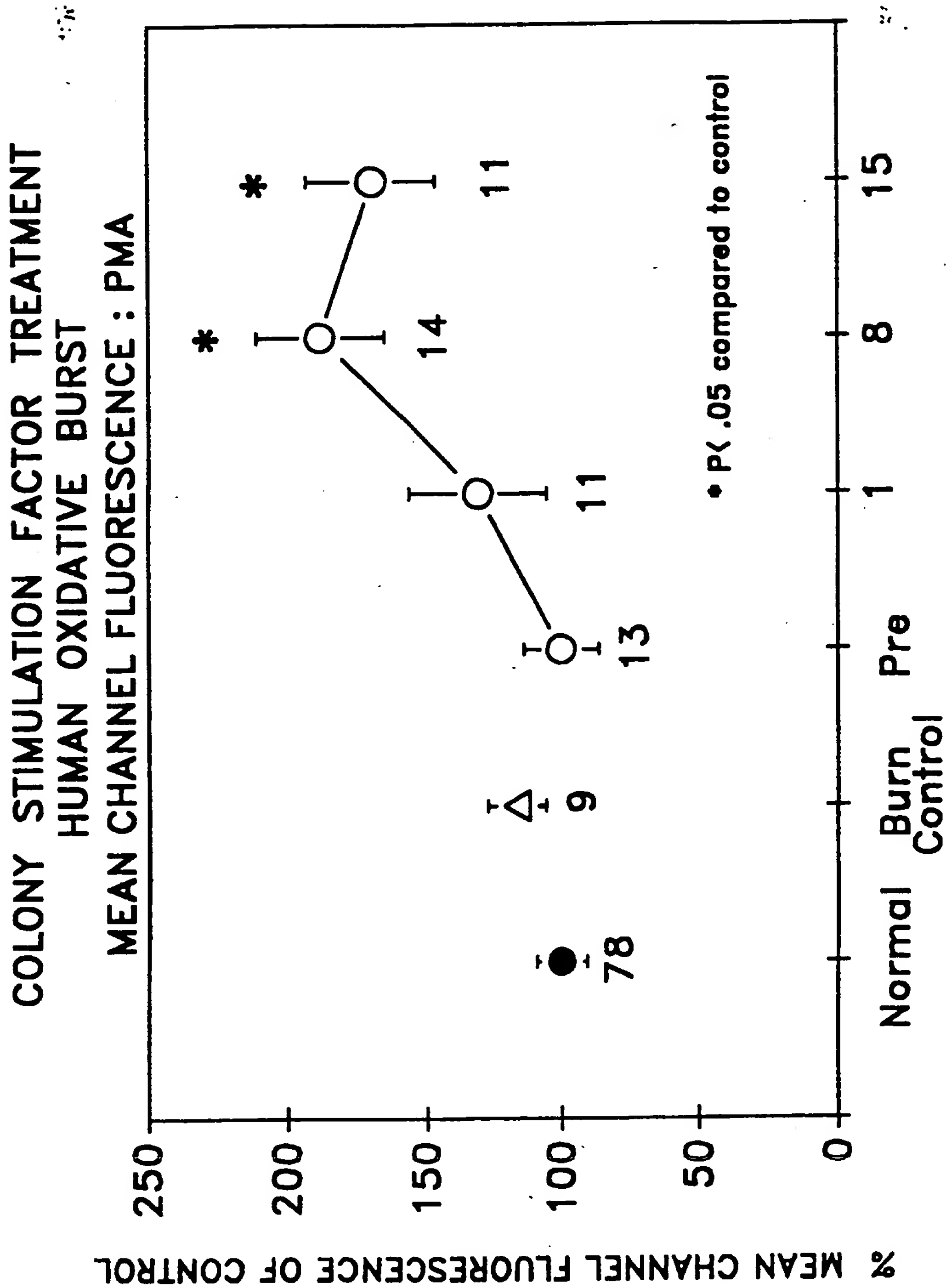
COLONY STIMULATION FACTOR TREATMENT
HUMAN OXIDATIVE BURST
MEAN CHANNEL FLUORESCENCE : PMA
% MEAN CHANNEL FLUORESCENCE OF CONTROL
250
200
150
100
50
0
78
9
13
11
14
11
* P< .05 compared to control
Normal
Burn Control
Pre
1
8
15

Figure 3

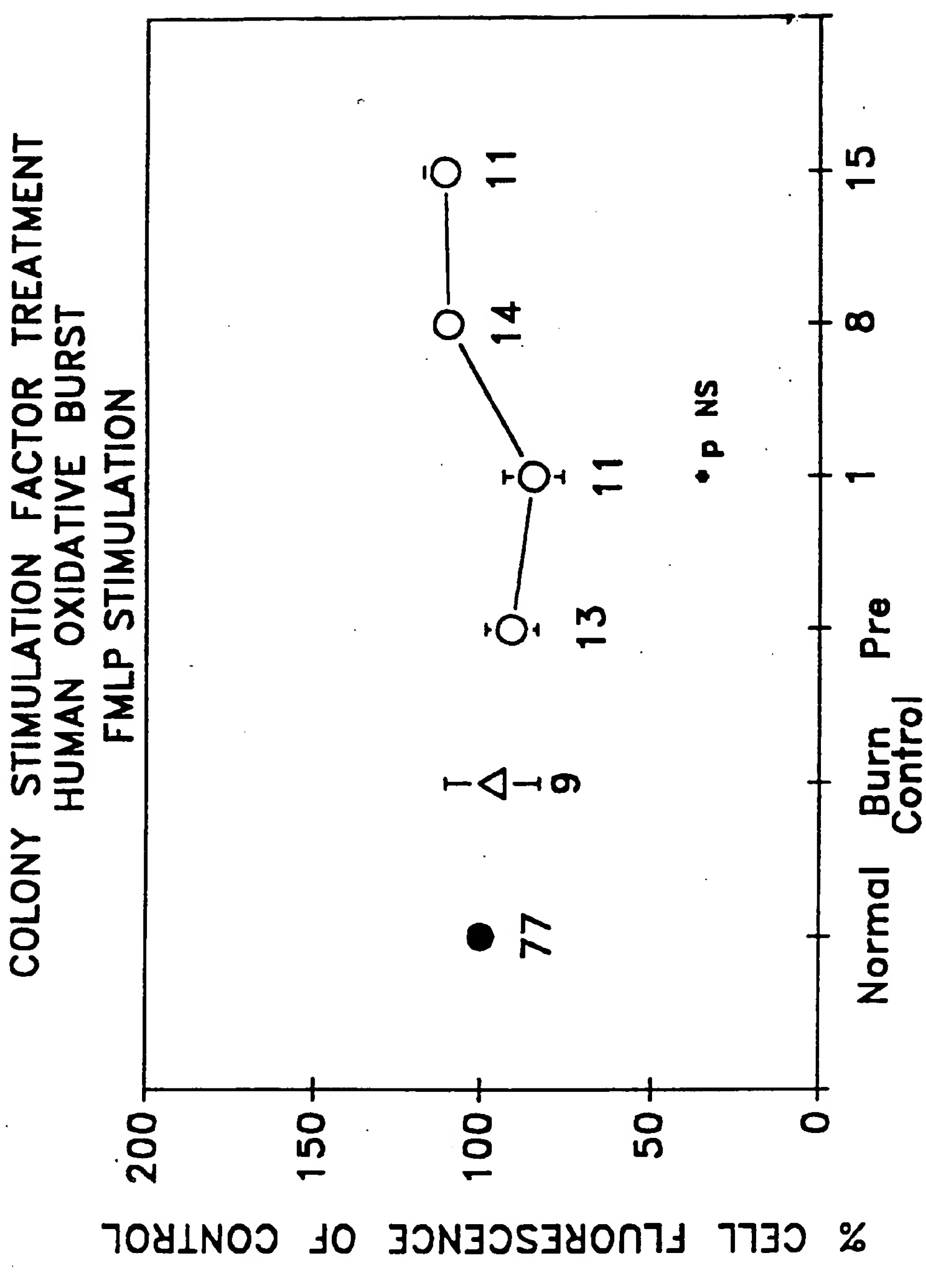
COLONY STIMULATION FACTOR TREATMENT
HUMAN OXIDATIVE BURST
FMLP STIMULATION
% CELL FLUORESCENCE OF CONTROL
200
150
100
50
0
Normal
Burn Control
Pre
1
8
15
77
9
13
11
14
11
* p NS

Figure 4

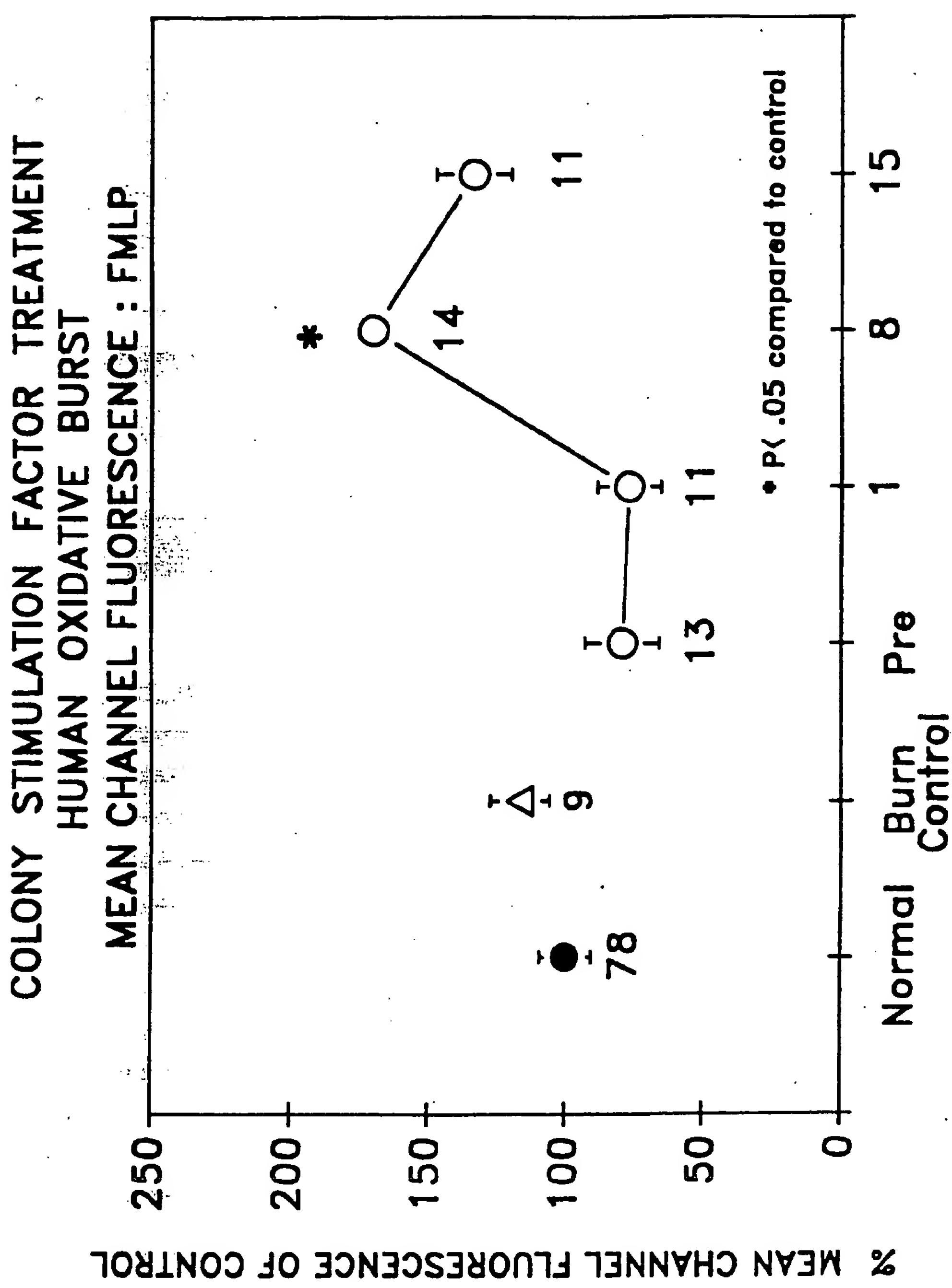
COLONY STIMULATION FACTOR TREATMENT
HUMAN OXIDATIVE BURST
MEAN CHANNEL FLUORESCENCE : FMLP
% MEAN CHANNEL FLUORESCENCE OF CONTROL
250
200
150
100
50
0
78
9
13
11
14
11
*
Normal
Burn Control
Pre
1
8
15
* P< .05 compared to control

Figure 5